Cutaneous properties of the Green and Golden Bell Frog *Litoria aurea*

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ABSTRACT

We measured rates of evaporative water loss (EWL) in *Litoria aurea* at 25, 30, 33, and 35°C. There was a substantial rise in EWL over this range of temperatures, averaging 12.3 and 23.5 mg g^{-1} h^{-1} at 25 and 35°C, respectively. The rise in EWL over this temperature interval was associated with a corresponding drop in cutaneous resistance to water vapour flux (r_c) which steadily declined from 6.9 to 3.5 s cm^{-1}. The average highest r_c measured for these eight frogs was 7 s cm^{-1} which places this species amongst frogs considered to have moderately waterproof skins. We also quantified the spectral reflectance of *L. aurea* dorsal skin at wavelengths between 280 and 900 nm. The skin had a peak at 550 nm which was expected for a green frog, but lacked the abrupt rise in reflectance at about 700 nm found in green leaves and some other species of green, leaf-sitting *Litoria* species. The absence of high near-infra-red reflectivity in *L. aurea* is attributed to its retention of melanin as a darkening pigment instead of pterorhodin which is used instead by green, leaf-sitting Litorine and Phyllomedusine frogs showing complete crypsis with green leaves.

INTRODUCTION

Amphibian skin serves many functions including respiratory gas exchange, excretion, antimicrobial activities, electrolyte and water uptake, water vapour barrier, and aposematic or cryptic colouration (Duellman and Trueb 1986). The skin of some species of Australian tree frogs is unusual amongst anurans in the latter two categories. Available data show *Litoria* species to have consistently higher cutaneous resistance to water vapour flux (r_c) than that considered typical for frogs (Withers *et al.* 1984; Buttemer 1990; Amey and Grigg 1995; Withers 1995). The r_c values of these frogs range from those found in some aquatic reptiles up to those associated with fully-terrestrial lizards (Buttemer 1990).

The other unusual property of Litorine skin is that some of the green, leaf-sitting species show very high near-infra-red reflectance (Cott 1940; Buttemer and Dain, unpublished manuscript). Because plants also have this pattern of reflectivity, such colour matching confers crypsis from predators capable of detecting near-infra-red radiation. Although *Litoria aurea* is also a green tree frog, it is typically found near permanent water and does not usually perch on broad, green leaves. This species presents an interesting opportunity to compare cutaneous characteristics of Australian green tree frogs from different habitats. Consequently, we have evaluated several adult *L. aurea* to see if they share the cryptic and water resistance properties of some of their green, leaf-sitting congeners.

METHODS

Experimental Animals

We used eight adult *L. aurea,* four males and four females for evaporative water loss measurements. Four of these frogs were collected at Homebush, New South Wales and four at Port Kembla, New South Wales. These were maintained in terraria with free access to water and were fed a diet of blowflies, mealworms, and cockroaches. Seven of these frogs gained mass and one maintained its body mass during their two-month captivity. The average body mass of the eight frogs was 19.1 ± 2.2 g (SE; range = 11.0 to 30.1 g). The single frog used for spectral reflectance evaluation was collected at Port Kembla.

Evaporative Water Loss Measurements

Rates of water loss at 25, 30, 33 and 35°C were measured gravimetrically using a flow-through chamber as described by Buttemer (1990). Frogs were placed in a plexiglas tube housed inside the chamber and were given about one hour to thermally equilibrate before measurements were recorded. Airflow into the chamber was maintained at a rate of 7 litres min^{-1} after first passing through a desiccant (Drierite). Air temperature was recorded just upstream from the frog using a calibrated thermocouple and humidity of air surrounding the frog was measured with a Vaisala humidity probe. The rates of evaporative water loss we report were recorded during the last hour of a frog's 2–2.5 hour exposure to a given temperature and are based on consecutive

10-minute measurements which are most representative of this period. We noted each frog's posture during these evaluations and recorded its body temperature by inserting a calibrated thermocouple into its cloaca within one minute of removing the frog from the chamber.

Cutaneous Resistance Evaluation

The rate of cutaneous water loss ($\dot{E}_c$) is driven by the vapour pressure gradient between the inside and outside of the skin and is restricted by two vapour resistance elements in series; the resistance of the skin to vapour flux (r_c) and the boundary layer resistance (r_b) associated with air entrapped around the outside surfaces of the animal (Spotila and Berman 1976). These relations can be expressed as:

$$\dot{E}_c = {}_sd_s\,(T_s) - RH\,{}_sd_a\,(T_a)/r_b + r_c \qquad (1)$$

where $\dot{E}_c$ is the rate of cutaneous water loss (g cm^{-2} s^{-1}), ${}_sd_s$ is the saturation vapour density (g cm^{-3}) of water in air at skin temperature (T_s), ${}_sd_a$ is the saturation vapour density (g cm^{-3}) at the air temperature (T_a) of the water loss measurement, RH is the measured air relative humidity (expressed fractionally), and r_b and r_c are boundary and cutaneous resistances to water vapour flux, respectively (both in s cm^{-1}).

Because the boundary layer resistance depends only on the physical geometry of the animal, it is possible to evaluate this term using an agar replica of a frog (Spotila and Berman 1976). We anaesthetized the smallest and largest frogs using tricaine methanesulfonate (MS 222) and made moulds of them in their water-conserving posture (Pough *et al.* 1983) using dental alginate impression material. We poured 3% agar into these moulds and determined rates of water loss and body temperatures at 30°C for these agar models under the same conditions as experienced by live frogs during their water loss measurements. Because the agar replicas behave as free-water surfaces and thus lack cutaneous resistance (Spotila and Berman 1976), r_b may be evaluated by simple rearrangement of equation 1. Boundary layer resistance values for the other frogs were interpolated from these two measurements according to their body mass relative to these two frogs.

We estimated body surface area exposed to air using an equation derived by McClanahan and Baldwin (1969) and modified for frogs in water-conserving postures by Withers *et al.* 1982 ($SA = 6.6\ mass^{0.56}$; SA = surface area in cm^2 and mass is in g). This equation was found to accurately describe exposed surface areas of *Litoria caerulea* and *L. chloris* (Buttemer 1990).

Cutaneous Spectral Reflectance

We measured reflectance of light from the dorsal surface of a mostly green, adult *L. aurea* using a Hitachi U–3410 spectrophotometer. This instrument was fitted with an integrating sphere which permitted accurate measurement of diffuse reflection of live animals. We measured cutaneous reflectance of light at wavelengths between 280 to 900 nm in increments of 5 nm. These measurements were part of a comparative study to identify cutaneous properties for light transmittance, reflectance, and absorbance in a range of *Litoria* spp. (Buttemer and Dain, unpublished manuscript). Skin samples of the species studied were subjected to a colorimetric test to identify the presence of pterorhodin pigments (Bagnara and Ferris 1975).

Unless stated otherwise, values presented in this paper are means plus or minus their SE, with number of animals and number of measurements denoted by N and n, respectively.

RESULTS AND DISCUSSION

Evaporative Water Loss and Cutaneous Resistance

Rates of evaporative water loss (EWL) in *L. aurea* increased substantially between 25 and 35°C (Fig. 1). The average EWL for frogs maintaining the water-conserving posture (Pough *et al.* 1983) at 25°C was 12.3 mg g^{-1} h^{-1} (± 1.3; N = 8) and at 35°C EWL averaged 23.5 mg g^{-1} h^{-1} (± 1.6; N = 8). These values are much higher than those measured by Buttemer (1990) for either *L. caerulea* or *L. chloris* (Fig. 1). Such differences in EWL are due partly to differences in surface to volume ratios between species of varying

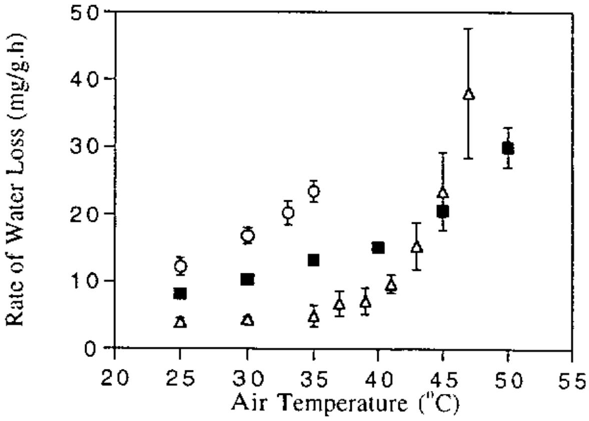

Fig. 1. The relation between rate of evaporative water loss and ambient air temperature in the Australian tree frogs *Litoria aurea* (open circles), *L. caerulea* (filled squares), and *L. chloris* (open triangles). The symbols designate the mean value recorded and the vertical line through each symbol describes the standard error of the mean. Values presented for *L. caerulea* and *L. chloris* are from Buttemer (1990).

mass. From size considerations alone, we would expect mass-specific EWL to be highest in the smallest species (*L. chloris*; mean mass = 18 g and *L. aurea*; mean mass = 19 g) and lowest in *L. caerulea* (mean mass = 49 g). Thus, the EWL of *L. aurea* and *L. caerulea* show the pattern expected, but *L. chloris* has a very low EWL up to temperatures as high as 40°C (Fig. 1).

Because EWL is due to physiological and morphological properties of the animal and to the physical conditions attending the water loss measurements, it is difficult to interpret the biological significance of the pattern and extent of variation in EWL between these species. However, because we quantified all of the variables needed to evaluate cutaneous resistance to water vapour flux (r_c; see equation 1), we can identify how this process varies between these species.

The boundary layer resistance (r_b) during EWL evaluations was 2.5 and 4 s cm^{-1} for the 10 g and 29 g *L. aurea*, respectively. We used these measurements as the basis for assigning r_b values to intermediately-sized frogs in our study. Accordingly, we rearranged equation 1 and solved for each frog's cutaneous resistance to water vapour flux (r_c) for all of our EWL evaluations.

In *L. aurea*, r_c declines steadily with rise in ambient temperature, from an average of 6.9 s cm^{-1} (± 0.5; N = 8) at 25°C to 3.5 s cm^{-1} (± 0.5; N = 8) at 35°C. This pattern is almost identical to that of *L. caerulea* over the same temperature range (Fig. 2; Buttemer 1990), however, the r_c values of *L. aurea* differ significantly from those of *L. caerulea* over this thermal interval (ANOVA F = 17.5; P = .0001). The pattern of r_c decline with temperatures above 25°C contrasts markedly with that of *L. chloris*. This species shows steady r_c when exposed to temperatures as high as 37°C (Fig. 2; Buttemer 1990), a pattern also shown by *L. xanthomera* (Thomas and Buttemer, unpubl. data). These differences imply greater thermal lability in blood flow to the surface and/or in rate of mucus discharge in *L. aurea* and *L. caerulea* than in the canopy-dwelling *L. chloris* and *L. xanthomera*.

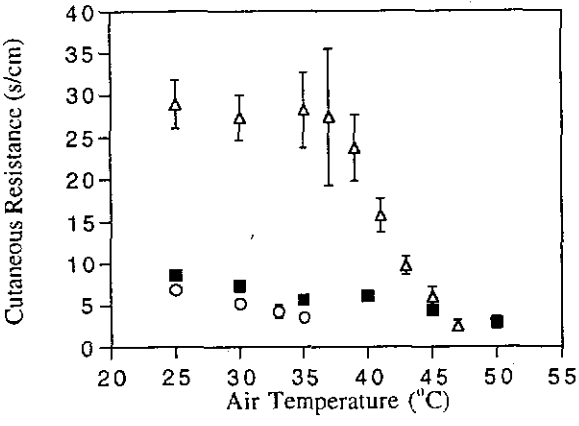

Fig. 2. Cutaneous resistance to water vapour flux as a function of air temperature in three species of Australian tree frogs. Symbols and vertical lines follow those described in Figure 1. Data for *L. caerulea* and *L. chloris* are from Buttemer (1990).

From the available information of r_c in *Litoria* species, it is clear that the species studied thus far have skin properties far different to those measured in ranid and bufonid frogs (Table 1). The latter species have skin which is highly permeable to water vapour and thus have r_c values approaching those of a free-water surface (r_c < 1 s cm^{-1}; Wygoda 1984). By contrast, the exposed skin of *Litoria* spp. while in the water-conserving posture have water vapour permeability's ranging between those of being moderately water-proof (7–40 s cm^{-1}) to those which are highly water proof (>100 s cm^{-1}). It is very interesting that *L. aurea* has an r_c as high as that of *L. caerulea* and *L. peronii*, species not as strongly linked to aquatic settings as *L. aurea* (Table 1).

Table 1. Average cutaneous resistance to water vapour flux (r_c) measured in selected anurans while in water-conserving postures. (Note: 2 s cm^{-1} was assumed for r_b of *L. gracilenta* and *L. rubella* and this value was deducted from total resistance values reported by Withers *et al.* 1984 and Withers 1995).

Species	r_c (s/cm)	Source of Data
Litoria gracilenta	115	Withers *et al.* 1984
L. chloris	39	Buttemer 1990
L. peronii	9	Amey and Grigg 1995
L. rubella	9	Withers 1995
L. caerulea	9	Buttemer 1990
L. aurea	7	Present Study
Rana catesbeiana	.05	Wygoda 1984
Bufo terrestris	.05	Wygoda 1984

The r_c of *L. aurea* would permit this species to attain higher body temperatures while basking and remain in sunlight longer before dehydrating than similar-sized ranid and bufonid species. Under the conditions of this experiment at 25°C, if *L. aurea* had an r_c like that evaluated in ranid and bufonid anurans (Table 1; Wygoda 1984), its EWL would have been 890 mg h^{-1} instead of the 235 mg h^{-1} that we measured. If we assume that *L. aurea* has a bladder capacity similar to other *Litoria* spp. (25% body mass; Main and Bentley 1964) and can tolerate similar levels of dehydration (Main and Bentley 1964; Johnson 1970), it could last 52 h in its water-conserving posture at 25°C but only 14 h if its r_c were the same as bufonid and ranid amphibia. It is important to recognize, however, that the ventral surface of *Litoria* spp. has a much lower r_c than the dorsal surface. Consequently, an active frog will expose more of its water-permeant underside and have a much greater EWL than when it is resting (Withers 1995).

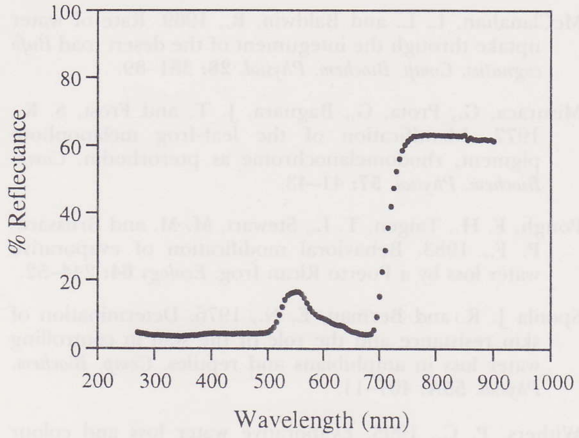

Fig. 3. Percentage of incident light reflected by an Umbrella Tree *Schefflera actinophylla* leaf as a function of wavelength of a monochromatic light source.

Cutaneous Spectral Reflectance

The green colour of plant leaves results from visible light being reflected mainly at 550 nm. Green leaves, however, reflect very highly at wavelengths just above 700 nm, a part of the spectrum considered outside the visible region for humans. This pattern is seen clearly in the spectral reflectance curve for an Umbrella Tree leaf *Schefflera actinophylla* (Fig. 3). Note especially the sharp transition of low to high reflectivity at 700 nm (Fig. 3). Such a pattern reduces transpirational water losses of leaves as most of the non-photosynthetically useful wavelengths are reflected and, thus, do not contribute to their solar heat load.

The significance of this spectral curve from the standpoint of crypsis in leaf-dwelling animals is that they would need to have the same spectral peak at 550 nm if their predators sensed wavelengths from 300 to just under 700 nm. However, if predators could sense wavelengths higher than 700 nm, then prey animals must have the same spectral rise at 700 nm as shown by chlorophyll-containing leaves if they are to remain camouflaged. There is suggestion that some predatory vertebrates can sense near-infra-red wavelengths (Cott 1940; Krempels 1988). Accordingly, it is quite striking that some green leaf-sitting frogs show the same pattern of rapid rise in reflectivity at wavelengths near 700 nm as shown by green leaves (Emerson *et al.* 1990).

Our measurements of spectral reflectance of some of the green leaf-sitting *Litoria* spp. shows striking similarity to that of green leaves, both in visible and at near-infra-red wavelengths (Fig. 4). The adult *L. aurea* we measured had a dorsal surface which was green to our eyes as attested to a reflectance peak near 530–550 nm as shown by two other green *Litoria* (Fig. 4). In contrast to the other two species, however, the *L. aurea* lacked a strong rise in reflectance at 700 nm (Fig. 4).

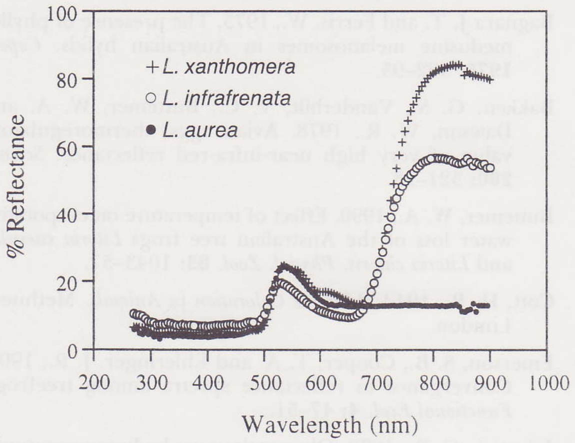

Fig. 4. Percentage of incident light reflected by three species of green Australian tree frogs as functions of wavelength of a monochromatic light source.

Besides the difference in near-infra-red reflectance between *L. aurea* and the green leaf-sitting *L. xanthomera* and *L. infrafrenata,* the latter two species tested positive for the presence of pterorhodin in their skin while *L. aurea* tested negative. We suggest that these differences in skin pigments may account for the disparate patterns of reflectivity shown by these frogs. Pterorhodin was first identified in the melanophores of some adult Phyllomedusine leaf-frogs (Bagnara *et al.* 1973). These species use this pigment as a means to darken their skin instead of melanin as found in most other vertebrates. Melanin absorbs strongly in the near-infra-red (Bakken *et al.* 1978) whereas pterorhodin is relatively transparent at wavelengths above 650 nm (Misuraca *et al.* 1976). Thus, frogs having pterorhodin as a darkening pigment for visible light can reflect most of the near-infra-red light reaching their highly-reflective iridophore layer. By contrast, frogs using melanin would absorb much of the near-IR wavelengths and, thus, show a much reduced reflectance of near-IR light. Interestingly, birds use non-melanin pigments to colour their eggs which also have very high near-infra-red reflectance (Bakken *et al.* 1978)

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